

# Significance of Pre-treatment Interferon-gamma Release in Patients With Non-small-cell Lung Cancer Receiving Immune Checkpoint Inhibitors

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**Abstract.** *Background/Aim:* We retrospectively investigated the significance of pre-treatment interferon-gamma release (IGR) as a biomarker for predicting the efficacy of immune checkpoint inhibitor treatment (ICI-tx). *Patients and Methods:* This study included non-small-cell lung cancer patients who received ICI-tx between January 1, 2016 and April 30, 2019. IGR was measured using the positive control of an enzyme-linked immunosorbent assay. We defined the pre-treatment cut-off level of IGR as 10 IU/ml. *Results:* Fifty-four patients were divided into two groups; those with an IGR  $\leq 10$  IU/ml (lower group: LG) ( $n=15$ ) and those with  $>10$  IU/ml (higher group: HG) ( $n=39$ ). The time to treatment failure (TTF) in the HG was significantly longer than that in the LG. In multivariate analyses, C-reactive protein and IGR levels were significant risk factors for TTF. *Conclusion:* Pre-treatment IGR level of  $>10$  IU/ml is recommended to identify those patients who will respond favourably to ICI-tx.

Several studies (1-3) have indicated that the programmed cell death 1 (PD-1)/PD-1 ligand 1 (PD-L1) axis and interferon-gamma (IFN- $\gamma$ ) are important for acquiring cellular immunity to *Mycobacterium Tuberculosis* (MTB). In a previous study (4), we measured the IFN- $\gamma$  release (IGR) levels in the phytohemagglutinin (PHA)-containing positive

control (PC) of the QuantiFERON<sup>®</sup>-TB Gold Plus (QFT-TBGP) enzyme-linked immunosorbent assay. We showed changes in IGR in the PC after immune checkpoint inhibitor treatment (ICI-tx), and demonstrated the potential of IGR as a biomarker. Furthermore, our subsequent study (5) demonstrated that patients with a PC IGR level  $>7.06$  IU/ml prior to ICI-tx had significantly longer progression-free survival than those with a PC IGR level  $\leq 7.06$  IU/ml. Huang *et al* (6). similarly reported that among non-small cell lung cancer (NSCLC) patients treated with chemotherapy, those with an IGR level  $>7.06$  IU/ml in the PC had significantly better survival than those with an IGR level  $\leq 7.06$  IU/ml. Further, Langan *et al*. (7) performed MTB screening in 70 patients who underwent immune checkpoint inhibitor therapy for metastatic skin cancer, and subsequently recommended MTB screening before ICI-tx.

In this study, we defined the IGR cut-off level in the PC prior to ICI-tx as 10 IU/ml. We enrolled patients with NSCLC who received ICI-tx alone, and retrospectively examined the difference in treatment efficacy between patients whose IGR levels were  $>10$  IU/ml and those with IGR levels  $\leq 10$  IU/ml. Furthermore, we assessed patients with positive QFT-TBG or QFT-TBGP tests to determine whether they developed MTB.

## Patients and Methods

*Ethics.* The study protocol was approved by the institutional review board of the Osaka Habikino Medical Center (formerly the Osaka Prefectural Medical Center for Respiratory and Allergic Diseases, Osaka, Japan) on May 5, 2020 (approval number: 1020). The requirement for informed consent was waived due to the retrospective study design and the anonymization of the data. All research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments. The details regarding our institution's opt-out policy are available at homepage of this institution (8).

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*Key Words:* Interferon-gamma release, immune checkpoint inhibitor, non-small-cell lung cancer, biomarker, time to treatment failure.

Table I. Patient demographics.

Variables	Overall	IGR (IU/ml) level		p-Value
		≤10 (n=15)	>10 (n=39)	
Gender				
Male	46	15	31	0.089
Female	8	0	8	
Age				
Mean (range)	69.0 (42-84)	68.8 (42-83)	69.0 (44-84)	0.923
Smoking status				
Ever	47	15	32	0.171
Never	7	0	7	
Performance status				
0-1 (0/1)	37 (4/33)	7 (0/7)	30 (4/26)	0.069
2-3 (2/3)	17 (15/2)	8 (6/2)	9 (9/0)	
Histology				
Adenocarcinoma	39	10	29	0.821
Squamous cell carcinoma	12	3	9	
Others	3	2	1	
Tuberculosis infection				
Latent or past	7	2	5	1.000
No	47	13	34	
Stage				
IIIB-C	8	2	6	0.747
IV	28	7	21	
Recurrence	18	6	12	
PD-L1 staining				
<1%	10	3	7	1.000
≥1%, <50%	14	4	10	
≥50%	23	7	16	
Unknown	7	1	6	
Driver mutation				
Yes	5	2	3	0.610
No	41	11	30	
Unknown	8	2	6	
Treatment Line				
First	22	8	14	0.390
Second	20	1	19	
Third or more	12	6	6	
ICI				
Pembrolizumab	23	7	16	0.946
Atezolizumab	8	2	6	
Nivolumab	23	6	17	
BMI (kg/m <sup>2</sup> )				
Mean (range)	22.7 (16.0-32.8)	20.6 (17.6-24.6)	23.5 (16.0-32.8)	0.009
Under weight (<18.5)	3	1	2	
Normal weight (≥18.5, <25.0)	38	14	24	
Pre-obesity or Obesity (≥25.0)	13	0	13	
Mean Laboratory data (range)				
Neutrophil-lymphocyte ratio	4.43 (1.05-19.21)	7.05 (2.00-19.21)	3.42 (1.05-8.28)	0.008
Serum albumin (g/dl)*	3.76 (2.5-4.8)	3.41(2.5-4.3)	3.89 (2.6-4.8)	0.012
C-reactive protein (mg/dl)	2.06 (0.03-14.79)	4.13 (0.07-14.79)	1.27 (0.03-6.22)	0.012

ICI: Immune checkpoint inhibitor; BMI: body mass index; IGR: Interferon-gamma release; PD-L1: programmed cell death ligand-1. \*Serum albumin levels were not examined in 2 patients with interferon-gamma levels >10 IU/ml.

*Patient selection.* One hundred and thirty-eight patients with NSCLC were treated with either nivolumab (N=102) or pembrolizumab (N=36) at our institution between January 1, 2016 and June 30, 2018. Of these, we selected 25 patients on whom QFT-

TBG or QFT-TBGP was performed for diagnosis of latent MTB within 14 days of ICI treatment. We also included 29 patients who were included in a previous study (4, 5) between July 17, 2018 and February 25, 2019. The total number of patients studied was 54.

**Baseline patient demographics.** The baseline patient demographics that we assessed before ICI treatment were sex, age, smoking status, Eastern Cooperative Oncology Group performance status (PS), body weight, height, body mass index (BMI), medications, disease complications, past illness and history of cancer treatment. BMI ( $\text{kg}/\text{m}^2$ ) was defined as the patient's weight in kilograms divided by the square of the patient's height in metres. Patients were categorized into the following three groups based on their BMI [according to established WHO categories (9)]: underweight ( $<18.5$ ); normal weight ( $18.5$ - $24.9$ ); and pre-obesity or obesity ( $\geq 25.0$ ). Blood and biochemical tests, performed within 14 days prior to ICI-tx, included: cell counts (white blood cells, lymphocytes, and neutrophils), serum albumin (s-Alb, g/dl), and C-reactive protein (CRP, mg/dl). The neutrophil-to-lymphocyte ratio (NLR) was calculated by dividing the neutrophil count by the lymphocyte count.

**Interferon-gamma release assay.** The IFN- $\gamma$  release assay (IGRA) was performed in-house with QuantiFERON<sup>®</sup>-TB Gold (QFT-TBG) (10) between 2016 and 2018 or with QFT-TBGP (11) between 2018 and 2019. The cut-off level of IGR in the PC was defined as 10 IU/ml, as described in the package inserts (12). Furthermore, the utility of the cut-off level of 7.06 IU/ml in the PC was reconfirmed, as established in our previous study (5). Latent tuberculosis or a past history of tuberculosis infection was determined based on the guidelines in the package inserts (12).

**Time to treatment failure and overall survival.** Time to treatment failure (TTF) was defined as the time between the administration of ICIs to their discontinuation for any reason, including progressive disease, treatment toxicity and death. Disease progression was determined by diagnostic imaging including computed tomography (CT), magnetic resonance imaging, bone scintigraphy and positron emission tomography, according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines version 1.1. The time of disease progression was considered as the day on which the CT was conducted. Overall survival (OS) was defined from the time of administration of ICIs to the date of mortality or last follow-up. TTF and OS data were last updated on March 31, 2020.

**Statistical analysis.** All analyses were conducted using the statistical software package R. The differences in variables between two groups were evaluated using the chi-square or Mann-Whitney *U*-test for categorical variables. TTF and OS were compared using the Kaplan-Meier method and log-rank test. Differences were considered statistically significant at  $p$ -values  $<0.05$ .

## Results

**Baseline patient demographics.** Fifty-four patients ( $n=54$ ) were included in this study, including those with IGR  $\leq 10$  IU/ml (lower group: LG) ( $n=15$ ), and those with IGR  $>10$  IU/ml (higher group: HG) ( $n=39$ ) in the PC of the QFT or QFT-plus assay before ICI-tx (Table I). There were no significant differences in clinical parameters between the groups. Table I shows that mean BMI and frequency of pre-obesity or obesity in the HG were significantly higher than those in LG ( $p=0.009$  and  $p=0.005$ , respectively). Contrastingly, NLR and CRP were significantly lower in the HG than in the LG ( $p=0.008$  and  $p=0.012$ , respectively). In

Table II. Analysis of overall response rate and disease control rate according to variables.

Variables	Number of patients	Response rate (%)	<i>p</i> -Value	Disease control rate (%)	<i>p</i> -Value
Total	54	11 (20.4)		20 (37.0)	
IGR (IU/ml) level					
$\leq 10$ IU/ml	15	1 (6.7)	0.153	3 (20.0)	0.129
$>10$ IU/ml	39	10 (25.6)		17 (43.6)	
Gender					
Male	46	11 (23.9)	$<0.001$	17 (37.0)	1.000
Female	8	0 (0)		3 (37.5)	
Age					
$\leq 70$	26	7 (26.9)	0.416	10 (38.5)	0.835
$\geq 71$	28	4 (14.3)		10 (35.7)	
Performance status					
0-1	37	9 (24.3)	0.293	18 (48.6)	0.014
2-3	17	2 (11.8)		2 (11.8)	
Histology					
Ad	39	7 (17.9)	0.737	14 (35.9)	0.972
non-Ad	15	4 (26.7)		6 (40.0)	
PD-L1 staining					
$<50\%$	25	4 (16.0)	0.849	10 (40.0)	0.542
$\geq 50\%$	22	4 (18.2)		6 (27.3)	
Stage					
IV	28	14.3	0.416	28.6	0.291
non-IV	26	26.9		46.2	
Treatment line					
1	22	22.7	0.989	31.8	0.710
$\geq 2$	32	21.9		40.6	
Body mass index					
$<22$ $\text{kg}/\text{m}^2$	27	5 (18.5)	1.000	9 (33.3)	1.000
$\geq 22$ $\text{kg}/\text{m}^2$	27	6 (22.2)		10 (40.7)	
Neutrophil-to-lymphocyte ratio					
$<3.6$	27	8 (29.6)	0.175	10 (37.0)	1.000
$\geq 3.6$	27	3 (11.1)		10 (37.0)	
Serum albumin					
$<4.0$ g/dl	27	5 (18.5)	0.886	7 (25.9)	0.173
$\geq 4.0$ g/dl	25	6 (24.0)		12 (48.0)	
C-reactive protein					
$<0.6$ mg/dl	27	8 (29.6)	0.175	15 (55.6)	0.009
$\geq 0.6$ mg/dl	27	3 (11.1)		5 (18.5)	

IGR: Interferon gamma release; PD-L1: programmed cell death ligand-1; Ad: adenocarcinoma.

contrast, s-Alb was significantly higher in the HG than in the LG ( $p=0.012$ ). s-Alb was not examined in two patients.

**Response to immune checkpoint inhibitor treatment.** The analysis of the response rate (RR) and disease control rate (DCR) by each variable (Table II) showed that RR in all patients was 20.4% and DCR in all patients was 37.0%. Males had significantly higher RR (23.9%) than females (0%) ( $p<0.001$ ). The DCR (48.6%) in patients with PS between 0-1 was significantly higher than that (11.8%) in those with a

Table III. Immune-related adverse events.

Number of patients with irAE (%)	Total (n=54)			IGR (IU/ml) level					
				≤10 (n=15)			>10 (n=39)		
Yes (%)	29 (53.7)			7 (46.7)			22 (56.4)		
No (%)	25 (46.3)			8 (53.3)			17 (43.6)		
Grade of irAE	1	2	≥3	1	2	≥3	1	2	≥3
Eruption	10	1	1	2	1	0	8	0	1
Thyroid dysfunction	7	0	0	3	0	0	4	0	0
Interstitial Pneumonitis	1	2	3	1	0	0	0	2	3
Colitis	1	3	1	0	0	1	1	3	0
Myositis	1	1	1	0	0	0	1	1	1
Renal dysfunction	0	3	0	0	0	0	0	3	0
Liver dysfunction	1	1	0	0	0	0	1	1	0
Others	0	2	0	0	1*	0	0	1**	0

irAE: Immune-related adverse events; IGR: interferon-gamma release. \*Grade 2 diabetes mellitus; \*\*Grade 2 adrenal dysfunction. No patient died of immune related adverse events.

PS between 2-3 ( $p=0.014$ ). The DCR (55.6%) in patients with CRP <0.6 mg/dl was significantly higher than that in patients with CRP ≥0.6 mg/dl (18.5%) ( $p=0.009$ ).

**Immune-related adverse events.** As shown in Table III, some degree of immune-related adverse events (irAEs) was observed in 29 (53.7%) of 54 patients. The most frequent irAE was eruption, observed in 12 patients (LG/HG: 3/9), thyroid dysfunction [7 patients (LG/HG: 3/4)], ICI-induced interstitial pneumonitis (ICI-IP) [6 patients (LG/HG: 1/5)], colitis [5 patients (LG/HG: 1/5)], myositis [3 patients (LG/HG: 0/3)], renal dysfunction [3 patients (LG/HG: 0/3)], and liver dysfunction [2 patients (LG/HG: 0/2)]. One patient experienced diabetes mellitus and one experienced adrenal dysfunction. No patients died of irAEs.

**Kaplan–Meier curves for time to treatment failure and overall survival.** HG patients had a significantly longer OS (483 days) as compared to LG patients (304 days; Figure 1A) ( $p=0.008$ ). Similarly, the median TTF in the HG (84 days) was significantly longer than that in LG (21 days; Figure 1B) ( $p=0.023$ ).

When an IGR cut-off level of 7.06 IU/ml was used (Figure 1C), the median OS in the HG (415 days) tended to be longer than that in the LG (291 days) ( $p=0.070$ ). Similarly, the median TTF was significantly longer in the HG (78 days) than in the LG (21 days; Figure 1D) ( $p=0.041$ ).

**Univariate analyses and multivariate analyses for time to treatment failure.** Of the 54 patients enrolled in this study, 40 patients discontinued ICI-tx due to disease progression, 9 due to irAEs; 1 refused ICI-tx, and 4 continued it. Amongst the 9 patients who discontinued ICI-tx due to irAEs, 5 experienced interstitial pneumonitis (G1/2/3: 1/1/3), 2

experienced G2 renal dysfunction, and 1 each experienced G3 colitis and G3 eruption, respectively. As shown in Table IV, in univariate analyses for TTF, PS ( $p=0.001$ ), stage ( $p=0.017$ ), NLR ( $p=0.025$ ), s-Alb ( $p=0.003$ ), CRP ( $p<0.001$ ), and IGR ( $p=0.023$ ) levels were all significant risk factors for ICI-tx failure. s-Alb was excluded from multivariate analysis because of missing data for 2 patients. In multivariate analyses, CRP and IGR levels were significant risk factors for TTF ( $p=0.008$  and  $p=0.041$ , respectively).

**Patients with a positive interferon-gamma release assay (IGRA).** In this study, 7 of 54 patients had a positive IGRA test. Amongst these patients, 6 were diagnosed with latent *MTB*, whereas 1 patient received anti-*MTB* treatment while receiving ICI-tx. None of these patients developed active *MTB* infection or recurrence of *MTB* infection.

## Discussion

In this retrospective study, we demonstrated that TTF and OS in the HG were significantly longer than those in the LG when the pre-ICI-tx IGR cut-off level of the positive control was set at 10 IU/ml. In multivariate analysis, CRP and IGR levels were identified as significant risk factors for TTF. The difference in TTF at an IGR cut-off of 10 IU/ml was clearer than that with a cut-off of 7.06 IU/ml. Therefore, based on our findings we recommend that 10 IU/ml should be used as the cut-off value to identify patients that will have a favourable response to ICI-tx.

Huang *et al.* (6) has previously reported that a higher pre-treatment PHA-stimulated IFN-γ response (high-PHA) was associated with better survival among advanced NSCLC patients treated with chemotherapy. These findings are similar to those of our previous study (5), in which we suggested that

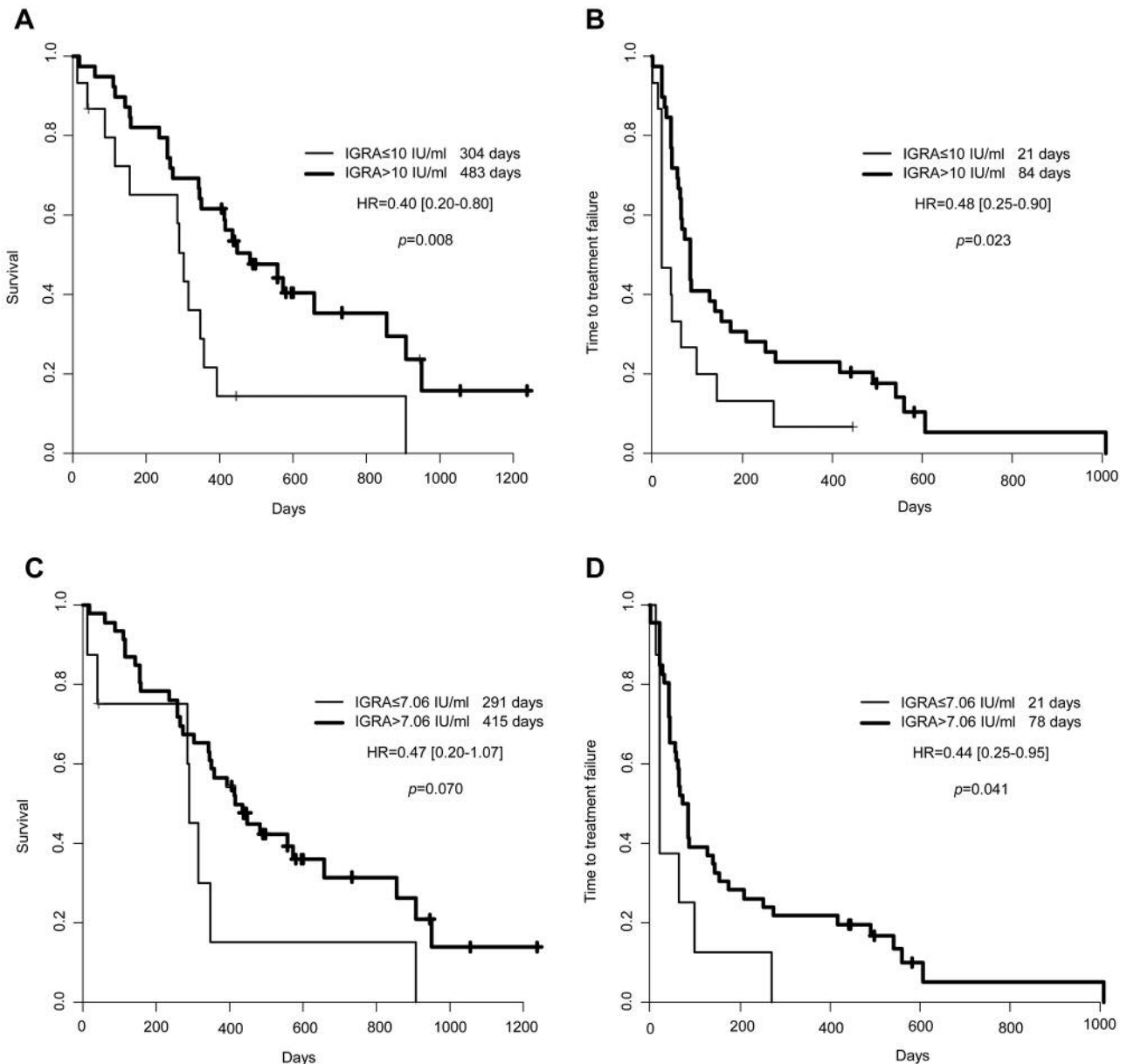


Figure 1. Time to treatment failure (TTF) and overall survival (OS) in patients with non-small-cell lung cancer using an interferon-gamma release (IGR) level cut-off of 10 IU/ml or 7.06 IU/ml prior to treatment with immune checkpoint inhibitors. Kaplan-Meier curves for TTF and OS indicate patients above the IGR cut-off level (in thick line) and those at or below the IGR cut-off level (thin line). A) The median OS in patients with IGR level >10 IU/ml (483 days) was significantly longer than that with IGR ≤10 IU/ml (304 days) [ $p=0.008$ , HR=0.40 (0.20-0.80)]. B) The median TTF (84 days) in patients with IGR level >10 IU/ml was significantly longer than that with IGR level ≤10 IU/ml (21 days) [ $p=0.023$ , Hazard ratio (HR)=0.48 (0.25-0.90)]. C) When using an IGR cut-off level of 7.06 IU/ml, the median OS of patients with IGR level >7.06 IU/ml (415 days) tended to be longer than that of patients with IGR level ≤7.06 IU/ml (291 days) [ $p=0.070$ , HR=0.47 (0.20-1.07)]. D) The median TTF in patients with IGR level >7.06 IU/ml (78 days) was significantly longer than that with IGR level ≤7.06 IU/ml (21 days) [ $p=0.041$ , HR=0.44 (0.20-0.95)].

high-PHA, which may reflect a better immunological status, is associated with improved progression-free survival.

Previous studies have identified several inflammation/nutrition biomarkers as prognostic factors for cancer. Overweight, in patients with colorectal cancer or nasopharyngeal

carcinoma, consistently shows favourable prognosis (13, 14). High NLR is also associated with an adverse OS in many solid tumours (15), whereas low baseline NLR is associated with favourable outcomes for metastatic melanoma patients treated with ipilimumab (16-18). CRP is an independent prognostic

Table IV. Univariate and multivariate analyses of risk factors for time to treatment failure.

	Univariate analysis HR (95%CI)	p-Value	Multivariate analysis HR (95%CI)	p-Value
Gender female	1.06 (0.47, 2.37)	0.888		
Age ≥72 years	0.81 (0.46, 1.43)	0.463		
Performance Status of 2-3	2.78 (1.49, 5.17)	0.001	1.56 (0.80, 3.04)	0.199
Histology of adenocarcinoma	0.91 (0.49, 1.69)	0.758		
PD-L1 staining ≥50%	1.21 (0.66, 2.24)	0.538		
Stage of IV	0.49 (0.28, 0.88)	0.017	0.59 (0.31, 1.12)	0.101
Treatment line of first line	0.66 (0.37, 1.17)	0.157		
Body mass index ≥25 kg/m <sup>2</sup>	0.85 (0.48, 1.51)	0.584		
Neutrophil-to-lymphocyte ratio ≥3.6	1.97 (1.09, 3.57)	0.025	1.52 (1.26, 4.82)	0.187
*Serum albumin (g/dl)	0.4 (0.22, 0.73)	0.003		
C-reactive protein ≥0.6 mg/dl	3.35 (1.80, 6.23)	<0.001	2.46 (1.26, 4.82)	0.008
IGR (IU/ml) level >10	0.48 (0.25, 0.90)	0.023	0.48 (0.24, 0.95)	0.041

IGR: Interferon gamma release; CI: confidence interval. \*Serum albumin was excluded from the multivariate analysis because of missing data for 2 individuals.

factor for survival in patients with advanced NSCLC receiving palliative first-line chemotherapy (19, 20). CRP/albumin ratio (CAR) is a potential independent predictor for disease progression and death in patients with operable NSCLC (21). In a meta-analysis (22), CAR was suggested as a potential prognostic marker in solid cancers.

In this study, IGR levels were significantly associated with high BMI, NLR, and CRP, and low s-Alb; therefore, using IGR as a biomarker would integrate these independent biomarkers. A high level of NLR and CRP, and a low level of s-Alb have been associated with systemic inflammation (23, 24). This study indicated that patients with chronic systemic inflammation would show poor outcomes for ICI-tx. Thus, IGR levels represent a potential biomarker to measure treatment efficacy in these patients.

Furthermore, IGR level is an improved biomarker, as compared to other biomarkers. Firstly, IGR level directly examines the activity of viable T cells; therefore, it is an index of immune function. Secondly, IGR levels change dynamically during ICI-tx, depending on the immune status of the patients as shown in our previous studies (4, 5). Thus, while other markers for ICI-tx efficacy are static biomarkers, IGR levels are dynamic, and more accurate.

Fujita *et al.* (25) reported that the incidence of active *MTB* in lung cancer patients receiving ICIs is 1.7%. They emphasized on monitoring patients with a positive IGRA test before ICI-tx for development of TB during immunotherapy. In the present study, 7 patients had a positive IGRA test. However, as of March 31, 2020, none of these patients had developed *MTB*. Close monitoring for future *MTB* development will continue, as suggested by Fujita *et al.* (25).

Our study has several limitations that must be considered. First, the sample size in this study was small. Second, the population of patients enrolled in this study was

heterogeneous. Finally, we did not examine cytokines and interleukins other than IFN- $\gamma$ .

To conclude, we recommend a pre-treatment IGRA level of 10 IU/ml as a cut-off value to identify patients who will respond favourably to receiving ICI-tx. IGR level is a dynamic biomarker that integrates inflammation/nutrition biomarkers such as BMI, NLR, CRP, and s-Alb. Close monitoring of *MTB* development is necessary in patients with a positive IGRA.

### Conflicts of Interest

T.H. received honoraria and research funding as a primary investigator at this institution from Ono Pharmaceutical Co. Ltd. (Osaka, Japan), Lilly Japan Co. Ltd. (Hyogo, Japan), AstraZeneca Co. Ltd. (Osaka, Japan), Taiho Pharmaceutical Co. Ltd. (Tokyo, Japan), Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan), and MSD Oncology Co. Ltd. (Tokyo, Japan). TH did not receive honoraria and research funding associated with this study. The other Authors have no conflicts of interest to declare in relation to this study.

### Authors' Contributions

All Authors were involved in the conception and design of the study, the acquisition of data, or the analysis and interpretation of data; drafting the article or revising it critically; and final approval of the version to be submitted. T.H., T.K., H.S., S.N., A.T, N.M., S.H., and N.O. collected clinical data. H.Y., A.M. and Y.T. performed measurement of QuantiFERON<sup>®</sup>-TB Gold Plus. H.K. performed blood examination. K.K made the pathological diagnoses. T.H., T.K., H.S., and T.T. performed the statistical analyses.

### Acknowledgements

The Authors wish to thank the patients and their families. The Authors also extend their appreciation to Mrs. Yuko Tani, who is

their secretary and summarized the data. The Authors thank Editage ([www.editage.jp](http://www.editage.jp)) for English language editing.

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*Received September 26, 2020*

*Revised October 10, 2020*

*Accepted October 12, 2020*